Wood Saponins. Part II. Further Studies on the Saponins from Morabukea* [Mora gonggrijpii (*Kleinh.*) *Sandwith*].

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Saponin mixtures isolated from different logs of morabukea may vary considerably in constitution. In the present investigation a product of very low galactose content has been examined. Acid hydrolysis gave oleanolic acid (37%) and a mixture of D-glucose (9 parts) and D-xylose (4 parts). Hydrolysis of the methylated material yielded methyl oleanolate and a mixture containing 2 : 3 : 4 : 6-tetra-, 3 : 4 : 6-tri-, and 2 : 4-di-*O*-methyl-D-glucose, and 2 : 3- and 2 : 4-di-*O*-methyl- and 2- and 3-*O*-methyl-D-xylose, with very small amounts of 2 : 3 : 4 : 6-tetra- and tri-*O*-methylgalactoses.

Degradation of the periodate-oxidised saponin with phenylhydrazine, followed by methylation and hydrolysis of the product, gave methyl oleanolate and 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-xylose.

These results are discussed and compared with previous findings.

Mora gonggrijpii, a large tree occurring in the Guianas, produces the heavy and durable commercial timber, morabukea. It is often very difficult to distinguish this wood from that of the related species mora (*Mora excelsa*), but the materials used in the present investigation and in Part I * were all definitely identified as morabukea by a study of the herbarium material.

In Part I * the results of a preliminary investigation of the saponins (SI) from the heartwood of morabukea were reported. It was shown that D-galactose constituted 8·4% of the carbohydrate residue, the other components being D-glucose and D-xylose. A further preparation (Farmer and Campbell, *Nature*, 1950, **165**, 237) of the saponin (SII) from a different log, however, gave on hydrolysis only very small amounts of galactose. The second saponin sample has now been examined in detail.

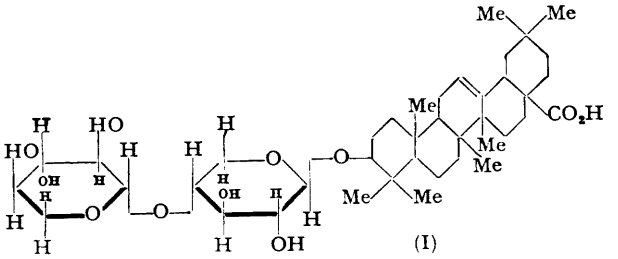
Hydrolysis of purified saponin SII gave oleanolic acid (37%) and a mixture of sugars. Quantitative paper chromatography of the latter (Hirst and Jones, *J.*, 1949, 1659) showed glucose (9 parts) and xylose (4 parts). The fully methylated saponin on hydrolysis yielded methyl oleanolate and a mixture of methylated sugars which were separated by partition on a column of cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511). The following materials were isolated : 2 : 3 : 4 : 6-tetra- (54·2%), 3 : 4 : 6-tri- (15·7%) and 2 : 4-di-*O*-methyl-D-glucose (2·1%), 2 : 3- (3·9%) and 2 : 4-di-*O*-methyl-D-xylose (2·3%), 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (*ca* 0·3%) and tri-*O*-methylgalactoses (0·7%), and a mixture (20·7%) containing 2- and 3-*O*-methyl-D-xyloses in the ratio of 1 : 2. The tetra- and tri-*O*-methylglucoses and 2 : 4-di-*O*-methyl- and 2-*O*-methyl-xylose were obtained as crystalline

* Part I, *J.*, 1954, 752.

sugars, and 2 : 3-di-*O*-methyl- and 3-*O*-methyl-xylose yielded the corresponding crystalline aniline derivatives. The syrupy 2 : 4-di-*O*-methylglucose was identified by its R_G value, methoxyl content, demethylation to glucose, and by the formation of formaldehyde on periodate oxidation. Demethylation of the fractions corresponding in R_G value to tetra- and tri-*O*-methylgalactoses yielded galactose along with some glucose; because of the small amount of material available, however, these were not examined further. The proportions of the individual constituents of the mono-*O*-methylxylose fraction were established from the equilibrium rotation in water of the derived lactone mixture (cf. Part I, *loc. cit.*). Traces of free xylose were also identified among the hydrolysis products, though it is probable that this substance is an artifact arising from incomplete methylation of the saponin or from demethylation during hydrolysis. Similar considerations may well apply to the relatively small amount of di-*O*-methylglucose isolated.

As with the sample SI, it is evident that SII is a mixture. While these two preparations have, in general, similar compositions they vary considerably in structural detail. Thus, the oleanolic acid content (37%) of the sample SII is higher than that of SI (30%), the ratios of 2- to 3-*O*-methylxylose obtained on hydrolysis of the methylated derivatives differ (1 : 1 from SI and 1 : 2 from SII) and the proportion of galactose residues in SII is only a small fraction of that found in SI. Fractionation of the mixture of methylated sugars obtained on hydrolysis of the methylated saponin SI yielded, among other products, 2 : 4 : 6-tri-*O*-methyl-D-glucose (9·2%) and a smaller fraction (3*a*; 0·5%) (Part I, *loc. cit.*) which was not further investigated but was probably 2 : 3 : 6- or 3 : 4 : 6-tri-*O*-methyl-D-glucose. The methylated material SII, on the other hand, furnished 3 : 4 : 6-tri-*O*-methyl-D-glucose (15·7%) with only very small amounts of other tri-*O*-methylglucoses. This sugar was obtained as the crystalline β-anomer and was identified by comparison with a synthetic specimen (Sundberg, McCloskey, Rees, and Coleman, *J. Amer. Chem. Soc.*, 1945, **67**, 1080) kindly given by Dr. G. H. Coleman. Its isolation is of particular interest, as structures involving 1 : 2-linked glucose residues are uncommon in Nature, though their presence in the hemicellulose of Iceland moss (Granichstädten and Percival, *J.*, 1943, 54), in crown-gall polysaccharide (Putman, Potter, Hodgson, and Hassid, *J. Amer. Chem. Soc.*, 1950, **72**, 5024), in sophoraflavonoloside (Rabaté, *Bull. Soc. chim. France*, 1940, **7**, 565; Freudenberg, Knauber, and Cramer, *Chem. Ber.*, 1951, **84**, 144), and in apiin (Hemming and Ollis, *Chem. and Ind.*, 1953, 85) has been established.

The saponin SII on periodate oxidation and degradation of the product with phenylhydrazine (Barry, *Nature*, 1943, **152**, 537; Barry and Mitchell, *J.*, 1954, 4020 and references therein) yielded a material which, on hydrolysis, gave oleanolic acid and xylose, along with small amounts of glucose. Hydrolysis of the methylated degraded saponin gave methyl oleanolate, 2 : 3-di-*O*-methyl-D-xylose, and 2 : 3 : 4-tri-*O*-methyl-D-xylose with small amounts of other derivatives. The low $[\alpha]_D$ of the acetate of the degraded saponin suggests that the xylose residues are joined by β-linkages and the fragment (I) must be present in at least one component of the SII mixture.



(I)

In contrast to makoré saponin which was shown (King, Baker, and King, *J.*, 1955, 1338) to have a 6-sulphato-ester group attached in the glucose residue, the saponin SII contains no sulphur.

The above results have demonstrated the constitutional variability of the saponin, and, while the general nature of the product is apparent, more precise information regarding its composition must await the development of efficient fractionation procedures; the methods

tried so far proved unsuccessful. Paper chromatography (cf. Dutta, *Nature*, 1955, **175**, 85; King *et al.*, *loc. cit.*) has, as yet, failed to give any clear-cut separation of the constituents.

Experimental

General experimental directions are given in Part I (*loc. cit.*). Paper partition chromatography was carried out on Whatman No. 1 filter paper, unless otherwise stated, and the following solvent systems (v/v; top layer) were used: (A) butanol–ethanol–water–ammonia (40 : 10 : 49 : 1); (B) benzene–butanol–pyridine–water (1 : 5 : 3 : 3); (C) ethyl acetate–acetic acid–water (3 : 1 : 3); (D) pentanol–acetic acid–water (4 : 1 : 5); and (E) benzene–ethanol–water (169 : 47 : 15). All qualitative chromatograms were run in the presence of authentic specimens of the appropriate sugars.

The saponin was prepared from a sample of the heartwood of morabukea as described earlier (Farmer and Campbell, *loc. cit.*) (Found: S, nil). Hydrolysis of the product with ethanolic hydrogen chloride yielded crystalline oleanolic acid, m. p. and mixed m. p. 303°.

The saponin (20 g.) gave an acetate (24·5 g.), $[\alpha]_D^{19}$ −13° (*c*, 1·0 in $CHCl_3$) (Found: Ac, 38·4%). These constants were unchanged by further acetylation. Attempts to fractionate the product by stepwise precipitation or solution were unsuccessful.

The acetate (9 .g) was treated with 0·4N-sodium hydroxide at room temperature and yielded a purified saponin (4·8 g.) which was used in the following investigations.

Hydrolysis with Sulphuric Acid.—The purified saponin was heated at 100° with N-sulphuric acid for 7 hr. and gave oleanolic acid (37·0%) which was removed. The filtrate was neutralised and evaporated to dryness. Examination of the residue on the paper chromatogram [solvents (B) and (C)] indicated the presence of glucose and xylose, with very small amounts of galactose. A quantitative estimation (Hirst and Jones, *loc. cit.*) gave glucose (9 parts) and xylose (4 parts).

Methylation.—Methylation of the acetate (10·5 g.) with methyl sulphate and sodium hydroxide followed by treatment with methyl iodide and silver oxide gave a fully methylated product (6·3 g.), $[\alpha]_D^{19}$ −10° (*c*, 2·01 in $CHCl_3$) (Found: OMe, 32·8%).

Methanolysis, Hydrolysis, and Fractionation.—The methylated saponin (5·8 g.) was heated with methanolic 3% hydrogen chloride (300 c.c.) under reflux for 5½ hr. and the solution was neutralised with silver carbonate, filtered, and evaporated to dryness. The residue was heated with N-sulphuric acid (200 c.c.) at 100° for 9 hr. (rotation constant). Methyl oleanolate (1·9 g.) was removed and after recrystallisation from methanol had m. p. and mixed m. p. 196° (Found: OMe, 6·0. Calc. for $C_{31}H_{50}O_3$: OMe, 6·6%). The filtrate was neutralised with barium carbonate, filtered, and evaporated to dryness. Extraction of the residue with boiling acetone gave a mixture of methylated sugars (3·6 g.). This mixture (3·10 g.) was fractionated on a column of cellulose (Hough, Jones, and Wadman, *loc. cit.*) with butanol–light petroleum (b. p. 100—120°) (2 : 3) saturated with water as eluant to give fractions (1) 1·4358 g., (2) 0·0134 g., (3) 0·5806 g., (4) 0·0168 g., (5) 0·0210 g., (6) 0·0787 g., and (7) 0·5041 g. (recovery, 2·6504 g., 85·5%).

Fractions (3) and (6) were mixtures. Fraction (3) was refractionated on a column of cellulose as above, to give (3*a*) 0·2734 g., (3*b*) 0·1153 g., and (3*c*) 0·0460 g. Separation of a portion of (3*b*) on 3MM paper (solvent A) gave (3*bi*) 0·0185 g. and (3*bii*) 0·0382 g. Fraction (3*bi*) was combined with (3*a*).

Fractionation of material (6) on 3MM paper as above gave fractions (6*a*) 0·0197 g. and (6*b*) 0·0268 g. The former was combined with fraction (5), and the latter with fraction (7).

Fraction (1). This had R_G 1·00 and crystallised completely to give 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 94°, $[\alpha]_D^{19}$ +98° (5 min.), +87° (1 hr.), +81° (24 hr., const.) (*c*, 1·1 in H_2O) (Found: C, 51·0; H, 8·5; OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%).

Fraction (2). This had the same R_G value as tetra-*O*-methylgalactopyranose. Demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) gave galactose and glucose, identified on the paper chromatogram (solvent B). The R_G value indicated that the glucose component was 2 : 3 : 4-tri-*O*-methylglucose.

Fraction (3a). The fraction was freed from traces of impurity by further chromatography on a column of cellulose as before, with butanol–light petroleum (b. p. 100—120°) (3 : 7) saturated with water as eluant. The product crystallised completely and on recrystallisation from ether had m. p. and mixed m. p. 95—96°, $[\alpha]_D^{18}$ +45° (5 min.), +50° (15 min.), +63° (1 hr.), +73° (4 hr.), +79° (24 hr., const.) (*c*, 0·985 in H_2O) (Found: C, 48·3; H, 8·0; OMe, 40·8. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%). Hypoiodite oxidation (Hirst, McGilvray, and Percival, *J.*, 1950, 1297) indicated 97% of tri-*O*-methylaldose. Complete methylation gave

tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 91°, R_G 1·00. Periodate oxidation in phosphate buffer (Bell, *J.*, 1948, 992) gave no formaldehyde, while oxidation with metaperiodate (Greville and Northcote, *J.*, 1952, 1945) yielded 0·98 equiv. of volatile acid. Repeated attempts to prepare a crystalline aniline derivative were unsuccessful.

The material was compared on the chromatogram with authentic 3 : 4 : 6-tri-*O*-methyl-D-glucose (αβ-mixture; kindly supplied by Dr. G. D. Greville), and showed identical behaviour in the three solvents used. In solvents A and D it travelled at the same rate as 2 : 3 : 6-tri-*O*-methylglucose and faster than 2 : 4 : 6-tri-*O*-methylglucose. In solvent E it moved 22·4 cm. while the 2 : 3 : 6-derivative travelled 18·0 cm. overnight.

Fraction (3*b*ii). This had the same R_G value as 2 : 3-di-*O*-methylxylose and $[\alpha]_D^{19}$ +25° (*c*, 0·48 in H_2O). The syrup (16·6 mg.) on periodate oxidation yielded formaldehyde, identified as the dimedone compound (22 mg.), m. p. and mixed m. p. 184°. The aniline derivative had m. p. 121—123° (Found: OMe, 22·7. Calc. for $C_{13}H_{19}O_4N$: OMe, 24·5%).

Fraction (3*c*). This travelled on the chromatogram at the same rate as 2 : 4-di-*O*-methylxylose and crystallised completely to give 2 : 4-di-*O*-methyl-β-D-xylose, m. p. and mixed m.p. 105° (Found: C, 47·0; H, 7·9; OMe, 33·4. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 34·8%).

Fraction (4). This gave on demethylation galactose and very small amounts of glucose identified on the paper chromatogram (solvent B).

Fraction (5). This had the same R_G value as 2 : 4-di-*O*-methylglucose (solvent D; travelled 27·3 cm.; both 2 : 3- and 3 : 4-di-*O*-methylglucose travelled 32·2 cm. in 3 days at 23°) (Found: OMe, 28·1. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). Demethylation yielded glucose only. The syrup, on periodate oxidation, gave formaldehyde, identified as the dimedone compound, m. p. and mixed m. p. 180°.

Fraction (7). The syrup (OMe, 17·2%) did not crystallise. Fraction (7) (0·4536 g.) was dissolved in ethanol and the solution was evaporated slowly to small volume in a vacuum-desiccator. The crystals which separated (37 mg.) had m. p. 138°, unchanged on admixture with 2-*O*-methyl-D-xylose (Found: C, 44·2; H, 7·5; OMe, 18·5. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). Evaporation of the residual solution gave a syrup (0·3727 g.) which was examined by paper ionophoresis (cf. Foster, *J.*, 1953, 982) on Whatman No. 1 filter sheet at 400 v in borate buffer (pH 9·97). Both 2- and 3-*O*-methylxylose were detected (the complexes travelled 7·8 and 13 cm. respectively in 6 hr.).

The syrup on treatment with aniline gave 3-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 137° (Found: OMe, 13·1; N, 6·1. Calc. for $C_{12}H_{17}O_4N$: OMe, 12·9; N, 5·9%).

Oxidation of the syrup with bromine yielded a syrupy lactone having $[\alpha]_D^{18}$ +73° (6 hr.), +69° (76 hr.), +55° (295 hr.), +50° (504 hr., const.) (*c*, 0·974 in H_2O) (13·56 mg. required 4·11 c.c. 0·0202N-sodium hydroxide for neutralisation. Calc. for $C_6H_{10}O_5$: 4·14 c.c.).

Treatment of the lactones with methanolic ammonia gave the corresponding amides. The syrupy mixture with alkaline hypochlorite and semicarbazide hydrochloride (Weerman, *Rec. Trav. chim.*, 1917, **37**, 16) yielded hydrazodicarbonamide, m. p. and mixed m. p. 256°.

Periodate Oxidation and Degradation with Phenylhydrazine.—Saponin (9 g.) was dissolved in water (400 c.c.), sodium metaperiodate (30 g.) was added, and the solution was kept in the dark for 8 days (periodate consumption complete). Excess of periodate was destroyed by the addition of ethylene glycol and the solution was dialysed in a Cellophane bag till free from iodate (1 week). The solution was evaporated to *ca.* 200 c.c. and used without further treatment for the degradation.

To the above solution phenylhydrazine (60 g.) was added, followed by sufficient acetic acid to clarify the solution. The mixture was left for 14 days at room temperature in the dark and was then heated for ½ hr. in a boiling-water bath. After 24 hr. the solid material was separated by filtration, washed quickly with water, ethanol, and ether and dried in a vacuum-desiccator (yield, 2·8 g.).

Acetylation of the Degradation Product.—The solid was acetylated with pyridine and acetic anhydride, and the acetate recovered by precipitation with water. It was dissolved in acetone, and the solution dried (Na_2SO_4), evaporated to small volume, and poured into light petroleum (b. p. 80—100°) to yield a precipitate (2·2 g.), $[\alpha]_D^{18}$ +8° (*c*, 0·5 in acetone) (Found: Ac, 26·2%). Evaporation of the residual solution gave a brown syrup (0·3 g.) which was not examined further.

Deacetylation.—Deacetylation of the saponin acetate. The acetate (0·55 g.) of the degraded saponin was dissolved in acetone (100 c.c.), and 0·4N-sodium hydroxide (100 c.c.) added. The solution was left overnight and then diluted to 1 l. with water. Ions were removed by columns of resins and the solution was evaporated to dryness. The residue was dissolved in ethanol, the

solution filtered, and the filtrate evaporated to dryness to give a syrup (0·41 g.). Hydrolysis of this material with N-sulphuric acid gave xylose and a small amount of glucose, identified on the paper chromatogram, along with an insoluble residue.

Methylation.—The acetate (1·6 g.) of the degraded saponin was methylated, first with sodium hydroxide and methyl sulphate and then with silver oxide and methyl iodide in the usual manner. This gave a syrupy methylated derivative (1·23 g.) (Found : OMe, 14·4%, unchanged on further methylation).

Hydrolysis and Fractionation.—The foregoing syrup (1·05 g.) was heated in methanolic 3% hydrogen chloride (150 c.c.) under reflux for 7 hr., and the solution was neutralised with silver carbonate and filtered. The filtrate was evaporated to dryness and the residue heated with N-sulphuric acid (200 c.c.) for 9 hr. at 100°. The brown tar was separated by filtration and, after several recrystallisations from methanol, yielded methyl oleanolate, m. p. and mixed m. p. 188—189°. The filtrate was neutralised with barium carbonate and evaporated to dryness to give a syrup (0·2 g.). Examination on the chromatogram (solvent A) indicated the presence of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methylxylose as the main constituents with small amounts of 2 : 4-di- and mono-*O*-methylxylose and tri-*O*-methylglucose. The mixture of methylated sugars was separated on a column of cellulose (16″ × 1″) as before to give fractions (*a*) 45·8 mg., (*b*) 54·8 mg., and (*c*) 11·8 mg.

Fraction (*a*). This had the same R_G value as 2 : 3 : 4-tri-*O*-methylxylose (solvents A, D, and E) and gave a pink spot with aniline oxalate. The brown syrup was purified by chromatography on sheets of Whatman No. 1 paper (solvent A), but the product did not crystallise. Rehydrolysis with N-sulphuric acid and examination of the product on the chromatogram revealed only one spot, showing the absence of contaminating methyl 2 : 3-di-*O*-methylxylosides in fraction (*a*).

Fraction (*b*). This was a mixture containing 2 : 3-di-*O*-methylxylose with small amounts of what appeared to be tri-*O*-methylglucose. The xylose derivative was separated by sheet chromatography with butanol–light petroleum (b. p. 100—120°) (3 : 7) saturated with water as solvent, and with aniline in ethanol yielded 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine, m. p. 110—112°; the mixed m. p. with an authentic specimen (m. p. 121—123°) was 118—120°.

Fraction (*c*). This had the same R_G value as 2- or 3-*O*-methylxylose and was not further investigated.

Paper Chromatography of Saponin SII.—Chromatograms were run with several solvents but in no case was separation satisfactory. The position of the saponin on the paper was established by spraying with the periodate and then with the borax–boric acid–potassium iodide–starch reagents of Metzenberg and Mitchell (*J. Amer. Chem. Soc.*, 1954, **76**, 4187), the saponin being revealed as a pale elongated spot against a blue background. The best results were obtained with Whatman 3MM paper and butanol–acetic acid–water (4 : 1 : 5, v/v; top layer) as solvent. By the use of reference strips the zone containing the saponin was located and cut out. This was divided laterally into two equal portions, and the saponin in each was eluted and hydrolysed. Both fractions yielded glucose and xylose in approximately the same proportions.

The faster-moving saponin could be freed from traces of free sugars by partition on a column of cellulose; the product obtained in this manner was a very pale, non-reducing amorphous solid.

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